Figure 1

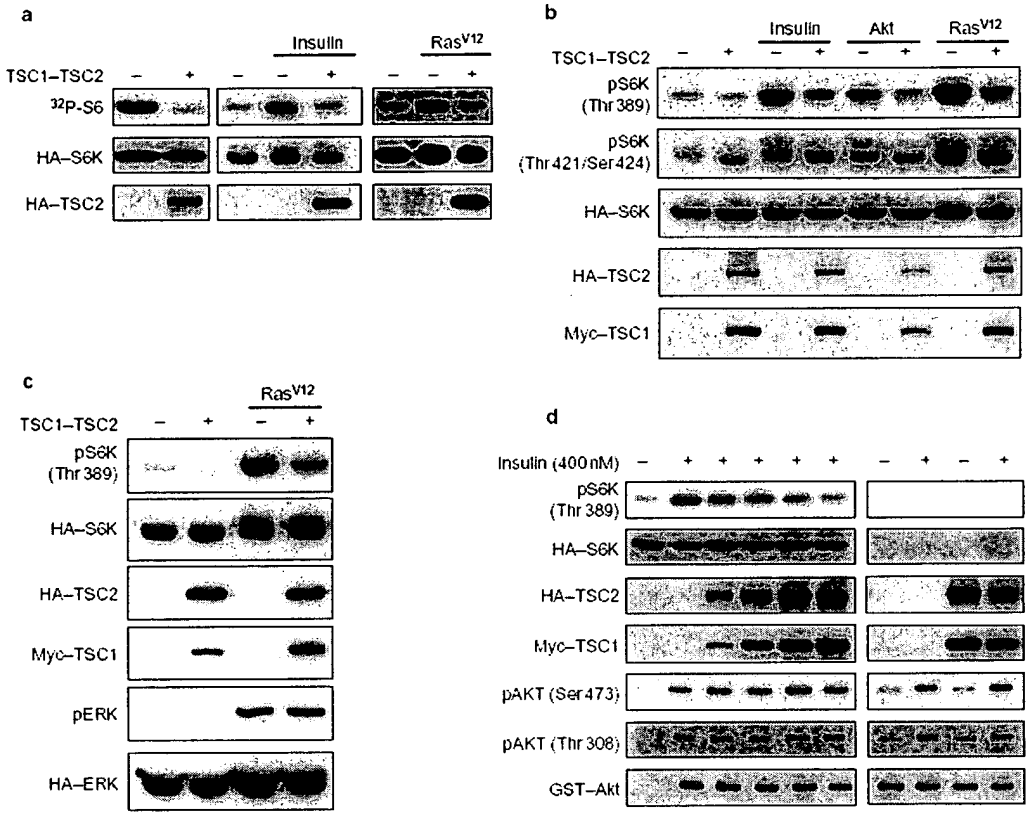

a
Insulin
RasV12
TSC1–TSC2
32P-S6
HA–S6K
HA–TSC2
b
Insulin
Akt
RasV12
TSC1–TSC2
pS6K (Thr389)
pS6K (Thr421/Ser424)
HA–S6K
HA–TSC2
Myc–TSC1
c
RasV12
TSC1–TSC2
pS6K (Thr389)
HA–S6K
HA–TSC2
Myc–TSC1
pERK
HA–ERK
d
Insulin (400nM)
pS6K (Thr389)
HA–S6K
HA–TSC2
Myc–TSC1
pAKT (Ser473)
pAKT (Thr308)
GST–Akt

Figure 2

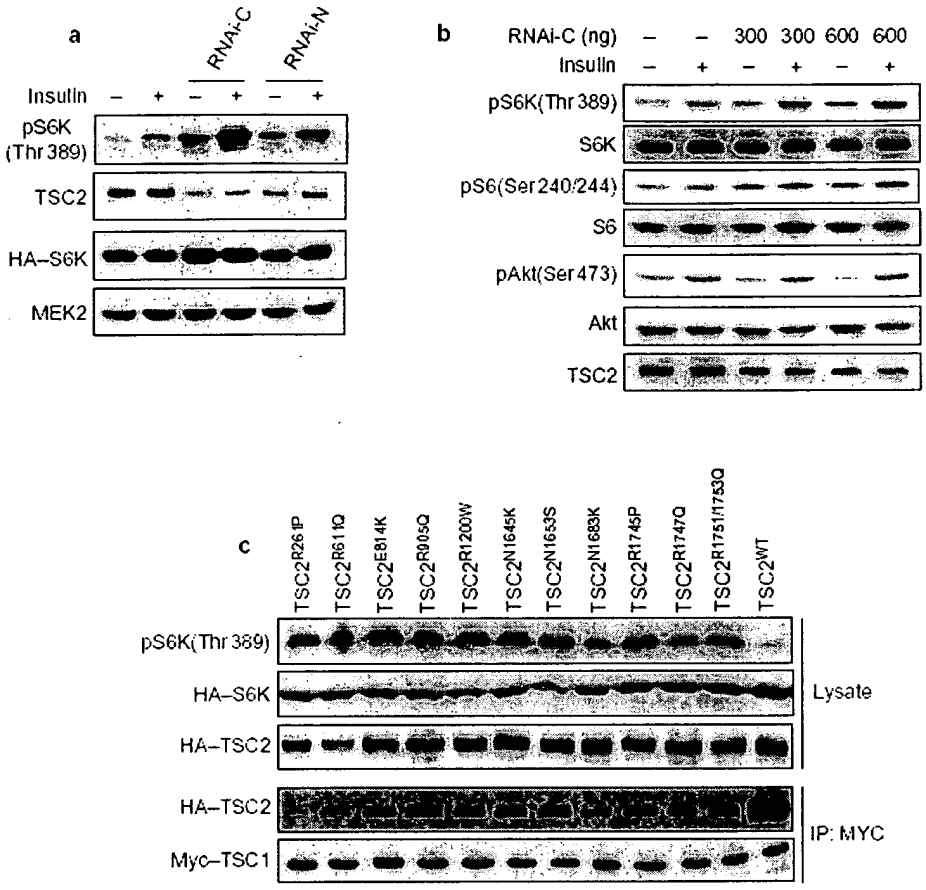
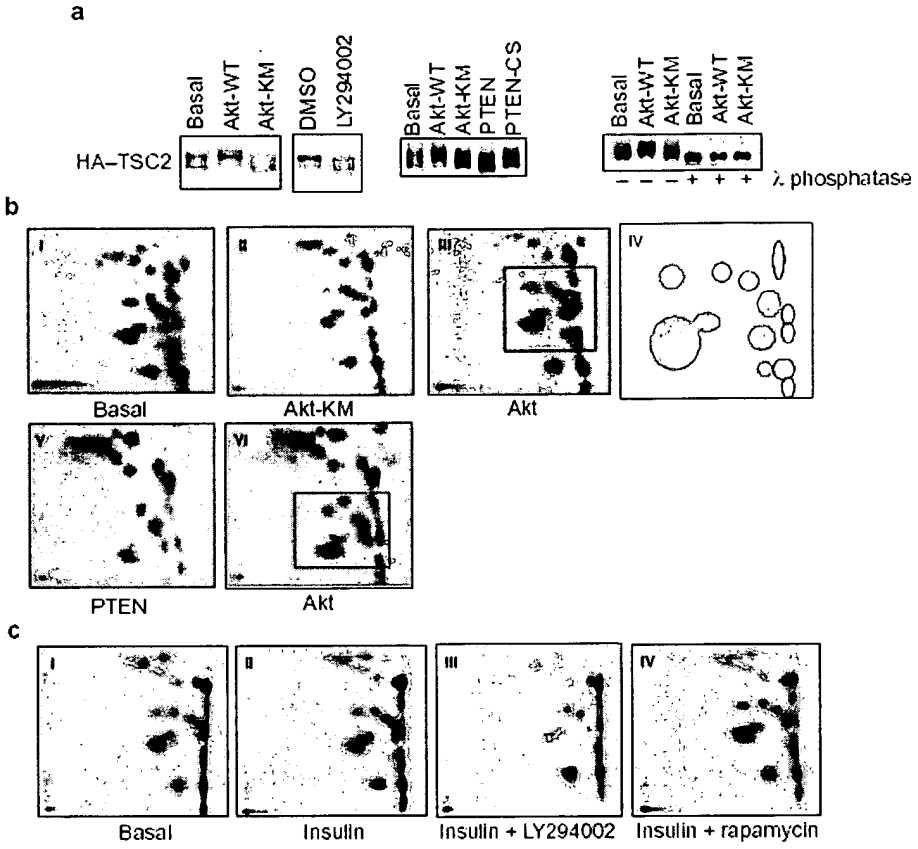

a
RNAi-C
RNAi-N
Insulin
pS6K (Thr389)
TSC2
HA–S6K
MEK2
b
RNAi-C (ng)
Insulin
pS6K(Thr389)
S6K
pS6(Ser240/244)
S6
pAkt(Ser473)
Akt
TSC2
c
TSC2R26IP
TSC2R611Q
TSC2E814K
TSC2R905Q
TSC2R1200W
TSC2N1645K
TSC2N1653S
TSC2N1683K
TSC2R1745P
TSC2R1747Q
TSC2R1751/1753Q
TSC2WT
pS6K(Thr389)
HA–S6K
HA–TSC2
Lysate
HA–TSC2
Myc–TSC1
IP: MYC

Figure 3


a
Basal
Akt-WT
Akt-KM
DMSO
LY294002
HA–TSC2
Basal
Akt-WT
Akt-KM
PTEN
PTEN-CS
Basal
Akt-WT
Akt-KM
Basal
Akt-WT
Akt-KM
– – – + + + λ phosphatase
b
I
II
III
IV
Basal
Akt-KM
Akt
V
VI
PTEN
Akt
c
I
II
III
IV
Basal
Insulin
Insulin + LY294002
Insulin + rapamycin

5

Figure 4

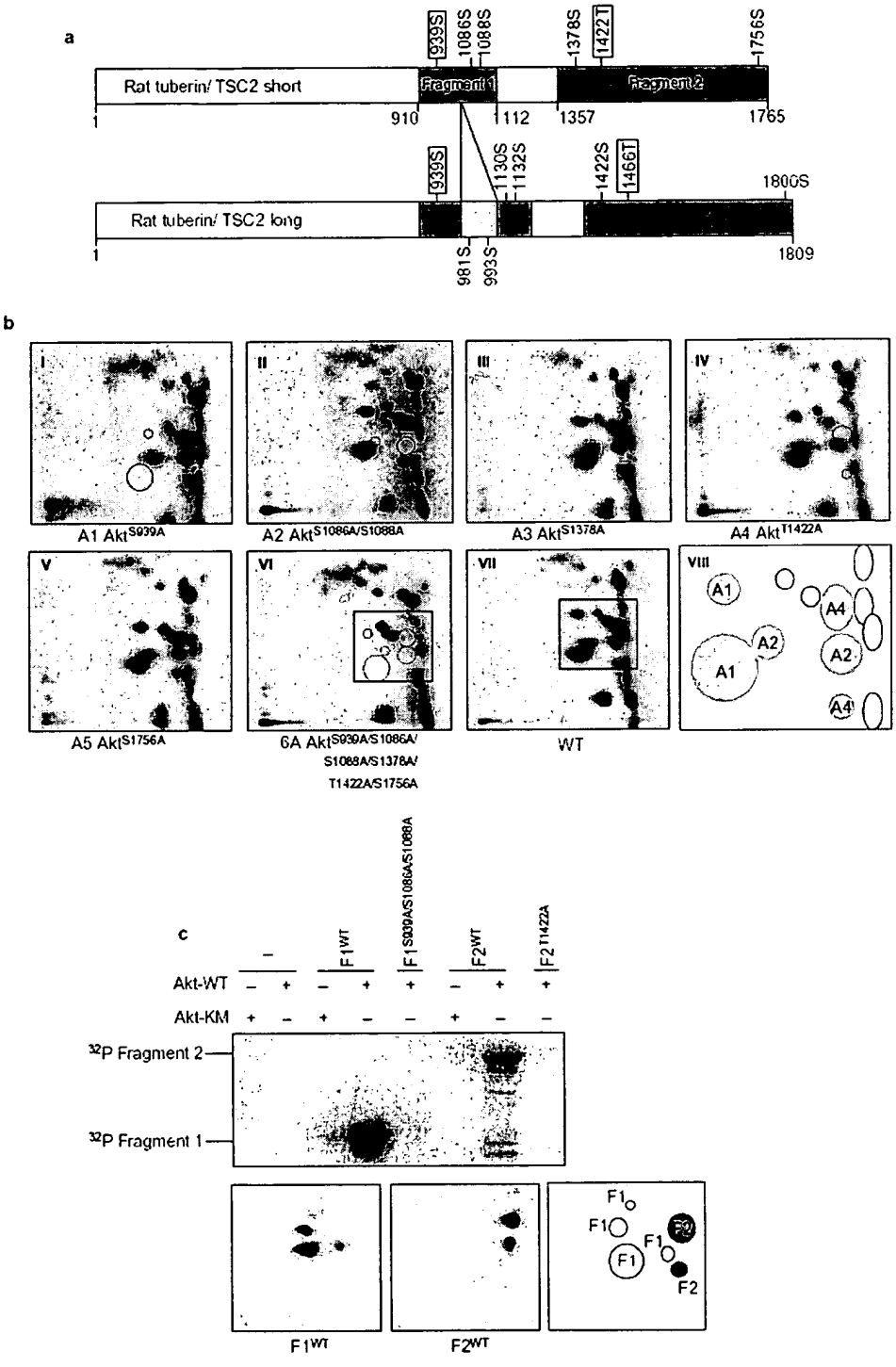

a
939S
1086S
1088S
1378S
1422T
1756S
Rat tuberin/ TSC2 short
Fragment 1
Fragment 2
1
910
112
1357
1765
939S
1130S
1132S
1422S
1466T
1800S
Rat tuberin/ TSC2 long
1
981S
993S
1809
b
I
II
III
IV
A1 Akt S939A
A2 Akt S1086A/S1088A
A3 Akt S1378A
A4 Akt T1422A
V
VI
VII
VIII
A5 Akt S1756A
6A Akt S939A/S1086A/ S1088A/S1378A/ T1422A/S1756A
WT
A1
A2
A4
c
F1 WT
F1 S939A/S1086A/S1088A
F2 WT
F2 T1422A
Akt-WT
Akt-KM
32P Fragment 2
32P Fragment 1
F1
F2
F1 WT
F2 WT

Figure 5

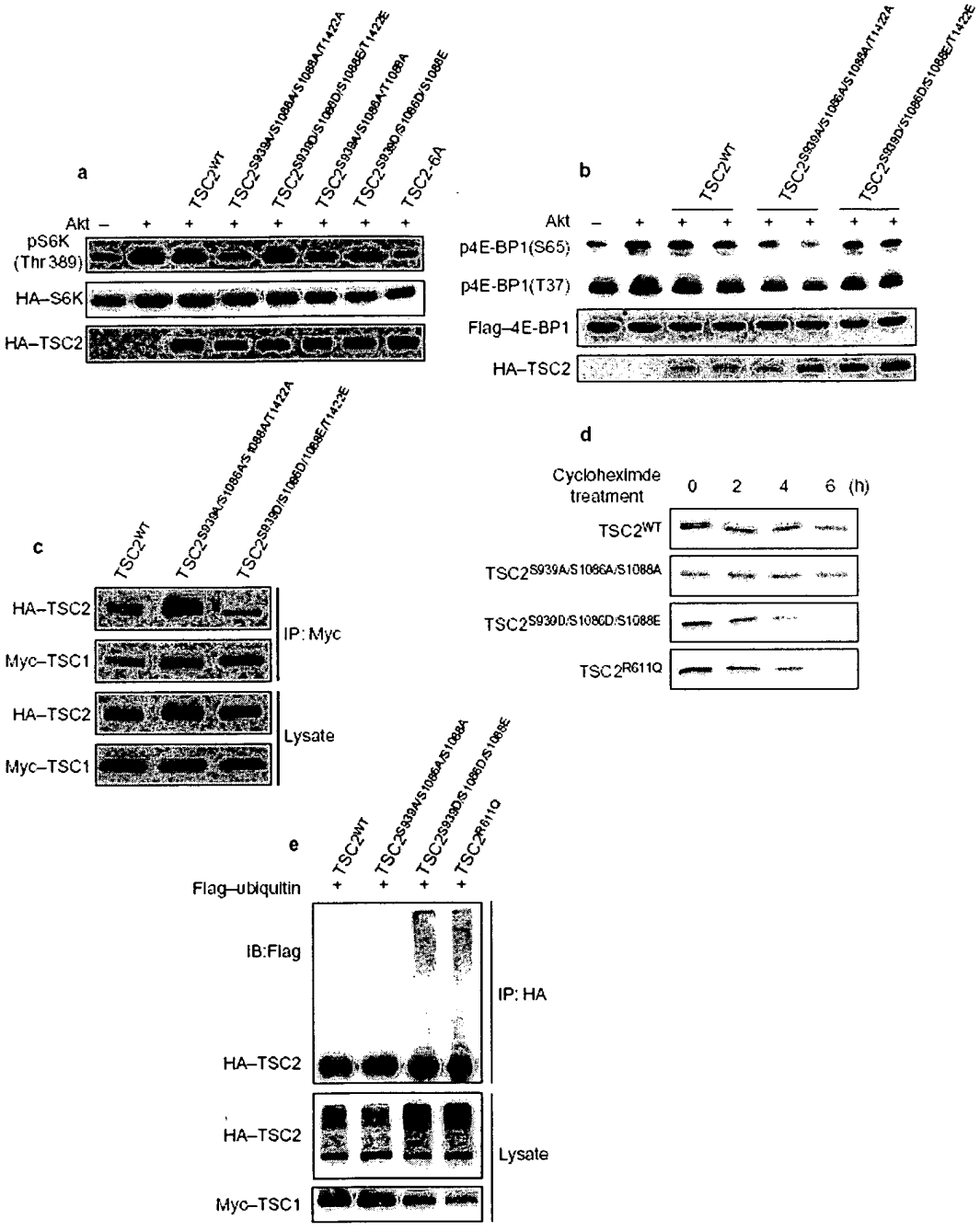

a
TSC2WT
TSC2S939A/S1086A/S1088A/T1422A
TSC2S939D/S1086D/S1088E/T1422E
TSC2S939A/S1088A/T1086A
TSC2S939D/S1086D/S1088E
TSC2-6A
Akt
pS6K (Thr389)
HA–S6K
HA–TSC2
b
TSC2WT
TSC2S939A/S1086A/S1088A/T1422A
TSC2S939D/S1086D/S1088E/T1422E
Akt
p4E-BP1(S65)
p4E-BP1(T37)
Flag–4E-BP1
HA–TSC2
c
TSC2WT
TSC2S939A/S1086A/S1088A/T1422A
TSC2S939D/S1086D/S1088E/T1422E
HA–TSC2
Myc–TSC1
IP: Myc
HA–TSC2
Myc–TSC1
Lysate
d
Cycloheximde treatment
0 2 4 6 (h)
TSC2WT
TSC2S939A/S1086A/S1088A
TSC2S939D/S1086D/S1088E
TSC2R611Q
e
TSC2WT
TSC2S939A/S1086A/S1088A
TSC2S939D/S1086D/S1088E
TSC2R611Q
Flag–ubiquitin
IB:Flag
IP: HA
HA–TSC2
HA–TSC2
Lysate
Myc–TSC1

Figure 6

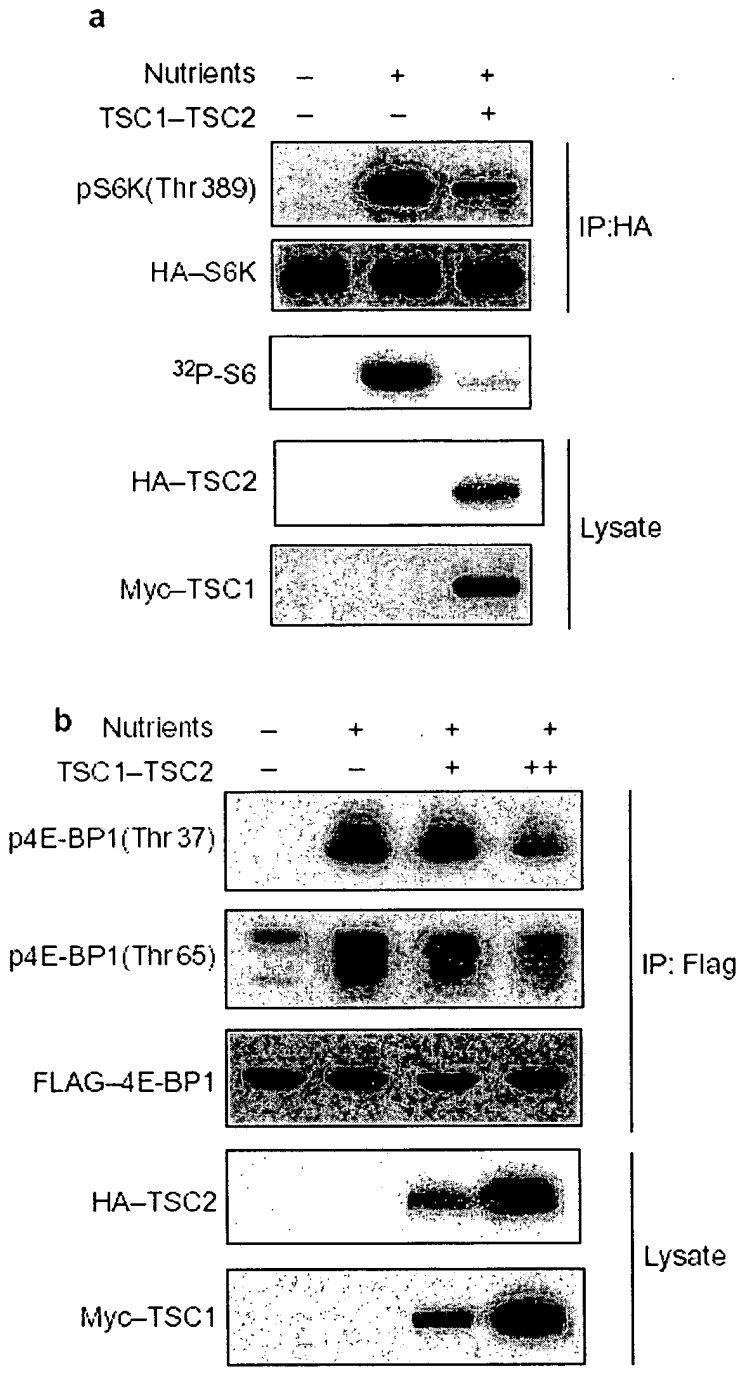

a
Nutrients – + +
TSC1–TSC2 – – +
pS6K(Thr389)
HA–S6K
IP:HA
32P-S6
HA–TSC2
Myc–TSC1
Lysate
b
Nutrients – + + +
TSC1–TSC2 – – + ++
p4E-BP1(Thr37)
p4E-BP1(Thr65)
FLAG–4E-BP1
IP: Flag
HA–TSC2
Myc–TSC1
Lysate

Figure 7

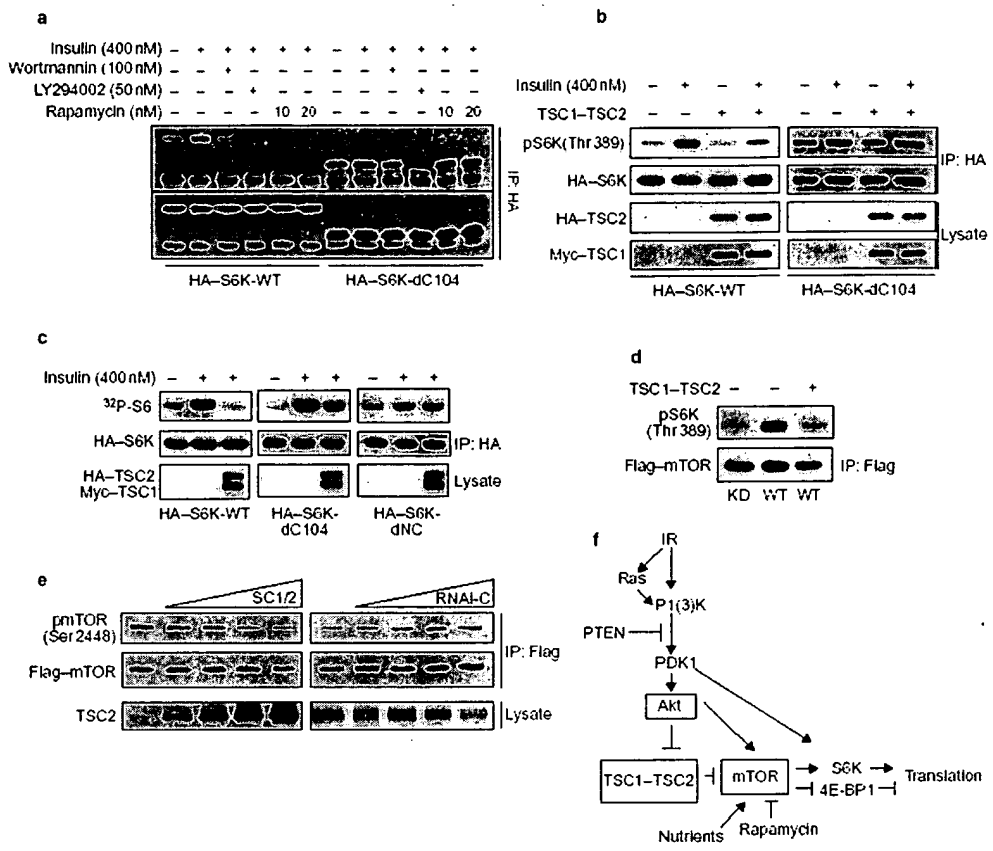

a
Insulin (400 nM)
Wortmannin (100 nM)
LY294002 (50 nM)
Rapamycin (nM)
10 20 10 20
IP: HA
HA–S6K-WT
HA–S6K-dC104
b
Insulin (400 nM)
TSC1–TSC2
pS6K(Thr389)
HA–S6K
HA–TSC2
Myc–TSC1
IP: HA
Lysate
HA–S6K-WT
HA–S6K-dC104
c
Insulin (400 nM)
32P-S6
HA–S6K
HA–TSC2
Myc–TSC1
IP: HA
Lysate
HA–S6K-WT
HA–S6K-dC104
HA–S6K-dNC
d
TSC1–TSC2
pS6K (Thr389)
Flag–mTOR
IP: Flag
KD WT WT
e
SC1/2
RNAi-C
pmTOR (Ser2448)
Flag–mTOR
TSC2
IP: Flag
Lysate
f
IR
Ras
P1(3)K
PTEN
PDK1
Akt
TSC1–TSC2
mTOR
S6K
4E-BP1
Translation
Nutrients
Rapamycin

Figure 8

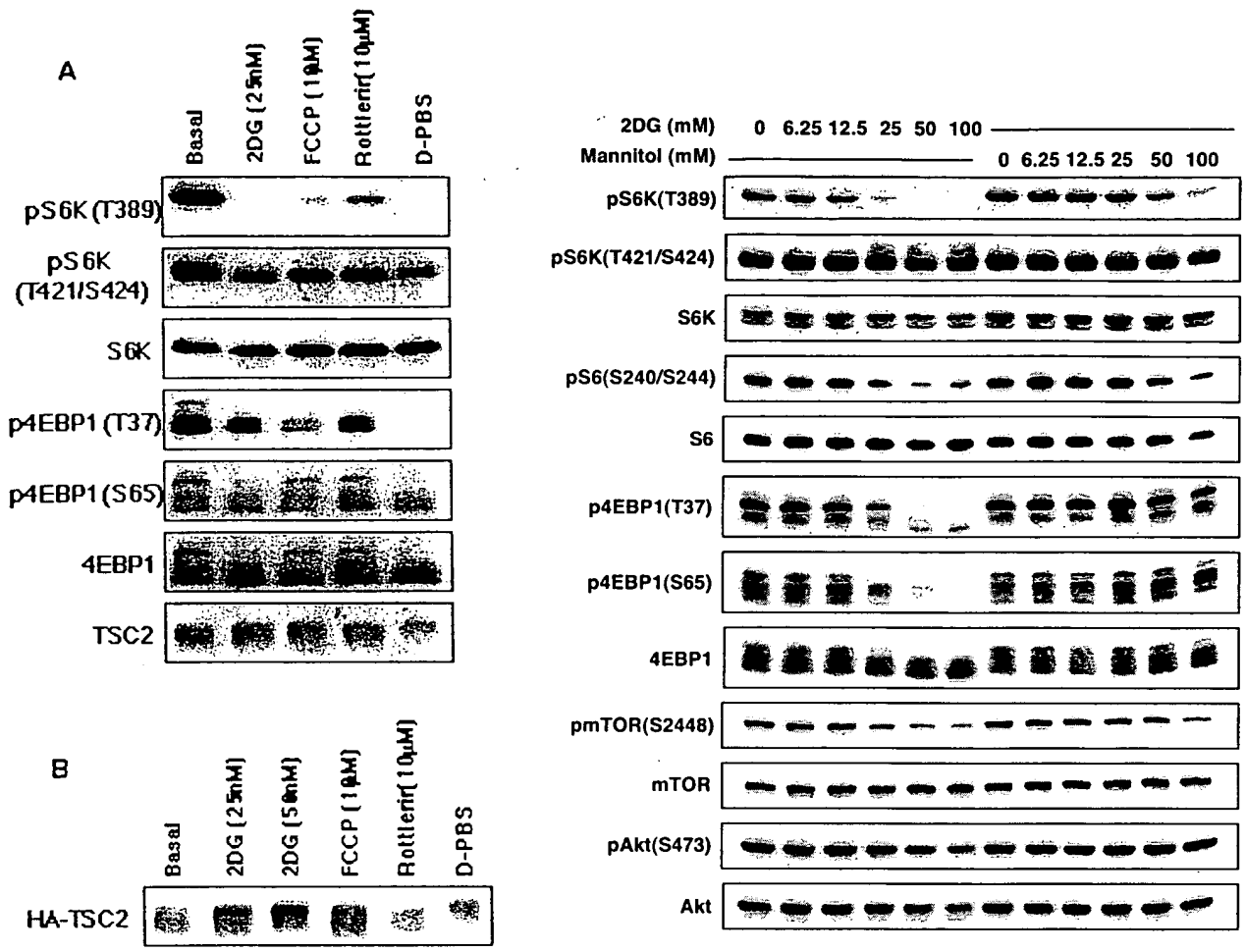

A
Basal
2DG (25mM)
FCCP (1μM)
Rottlerin(10μM)
D-PBS
pS6K (T389)
pS6K (T421/S424)
S6K
p4EBP1 (T37)
p4EBP1 (S65)
4EBP1
TSC2
B
Basal
2DG (25mM)
2DG (50mM)
FCCP (1μM)
Rottlerin(10μM)
D-PBS
HA-TSC2
2DG (mM) 0 6.25 12.5 25 50 100
Mannitol (mM) 0 6.25 12.5 25 50 100
pS6K(T389)
pS6K(T421/S424)
S6K
pS6(S240/S244)
S6
p4EBP1(T37)
p4EBP1(S65)
4EBP1
pmTOR(S2448)
mTOR
pAkt(S473)
Akt

Figure 8 continued

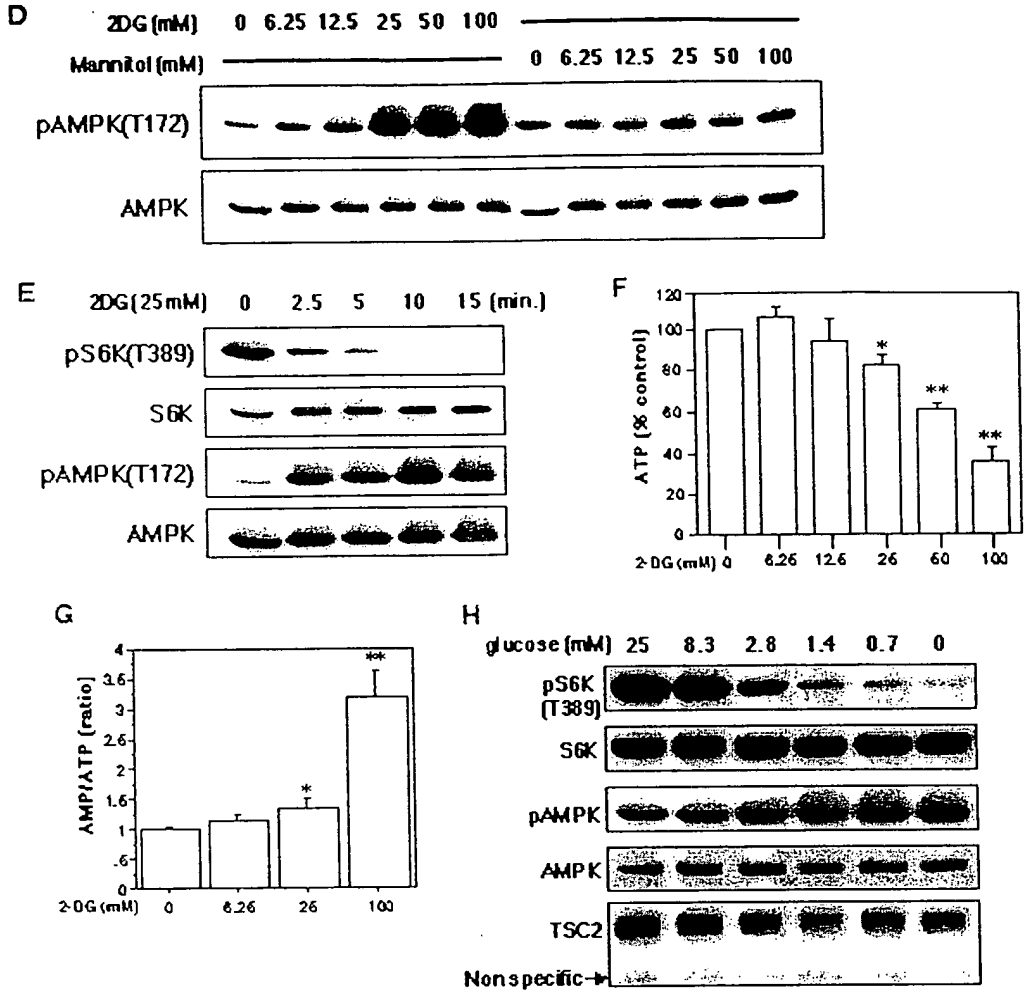


Figure 9

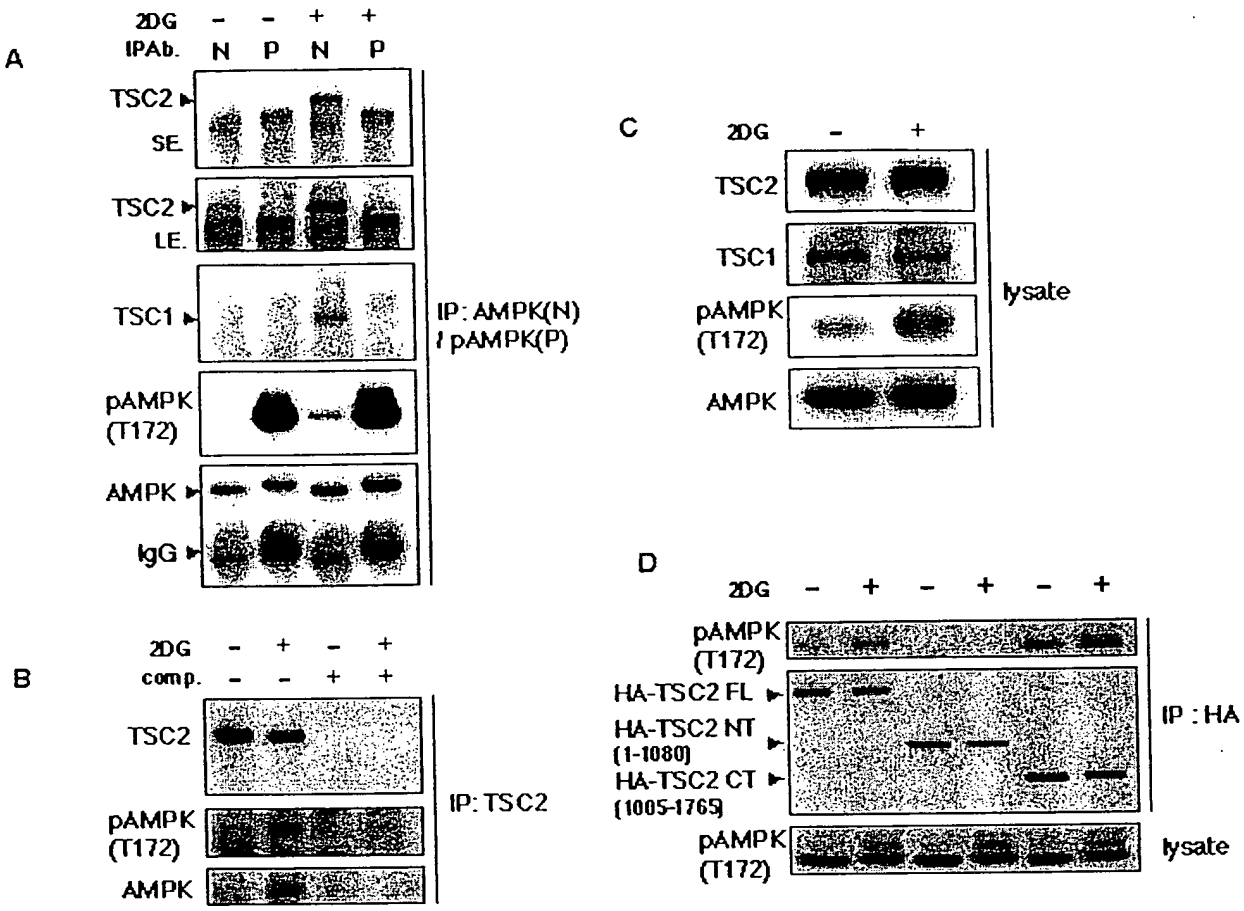

A
2DG - - + +
IPAb. N P N P
TSC2
SE.
TSC2
LE.
TSC1
pAMPK (T172)
AMPK
IgG
IP: AMPK(N) / pAMPK(P)
B
2DG - + - +
comp. - - + +
TSC2
pAMPK (T172)
AMPK
IP: TSC2
C
2DG - +
TSC2
TSC1
pAMPK (T172)
AMPK
lysate
D
2DG - + - + - +
pAMPK (T172)
HA-TSC2 FL
HA-TSC2 NT (1-1080)
HA-TSC2 CT (1005-1765)
IP : HA
pAMPK (T172)
lysate

Figure 10

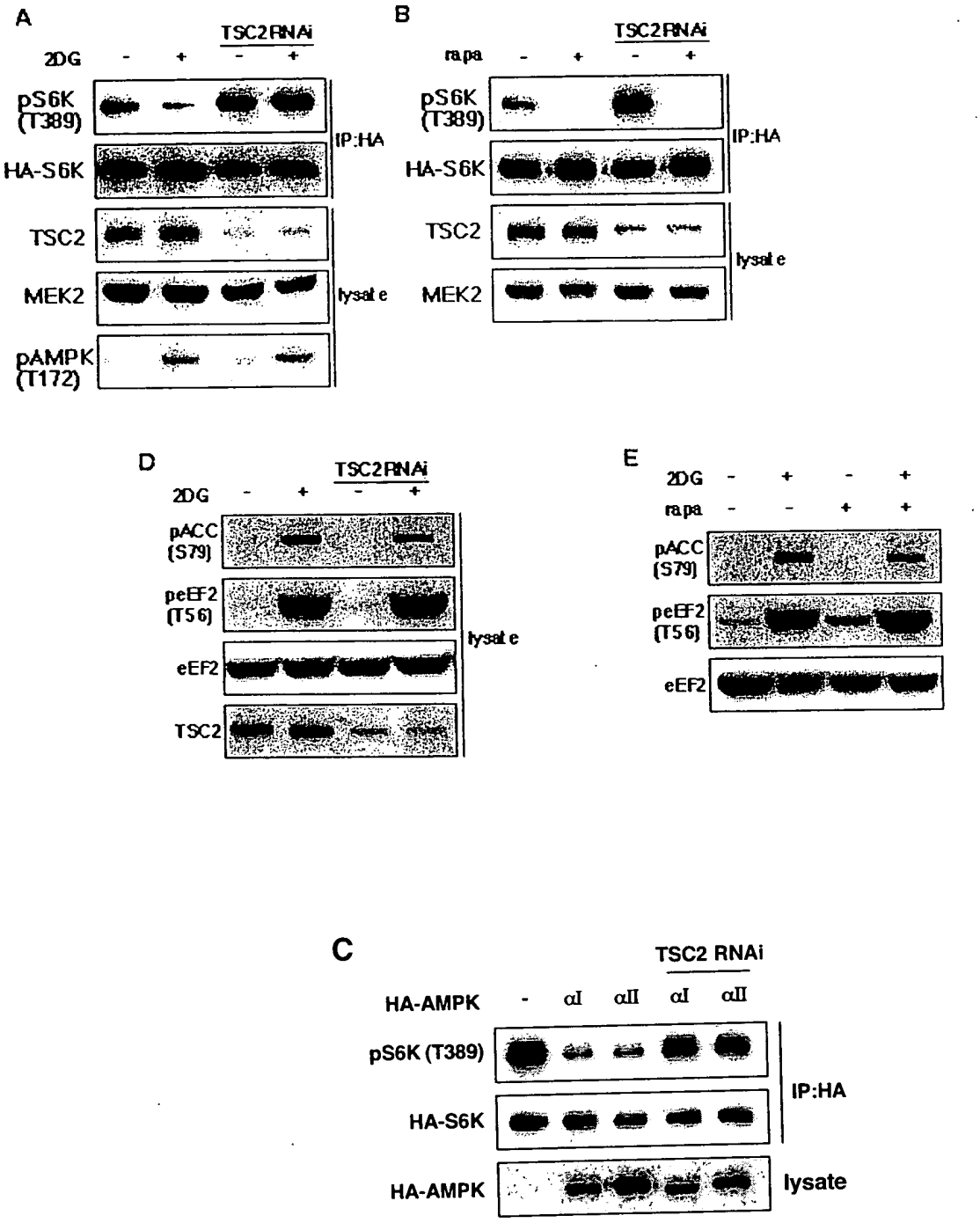

A
TSC2 RNAi
2DG - + - +
pS6K (T389)
HA-S6K
IP:HA
TSC2
MEK2
lysate
pAMPK (T172)
B
TSC2 RNAi
rapa - + - +
pS6K (T389)
HA-S6K
IP:HA
TSC2
MEK2
lysate
D
TSC2 RNAi
2DG - + - +
pACC (S79)
peEF2 (T56)
eEF2
TSC2
lysate
E
2DG - + - +
rapa - - + +
pACC (S79)
peEF2 (T56)
eEF2
C
TSC2 RNAi
HA-AMPK - αI αII αI αII
pS6K (T389)
HA-S6K
IP:HA
HA-AMPK
lysate

Figure 10 continued

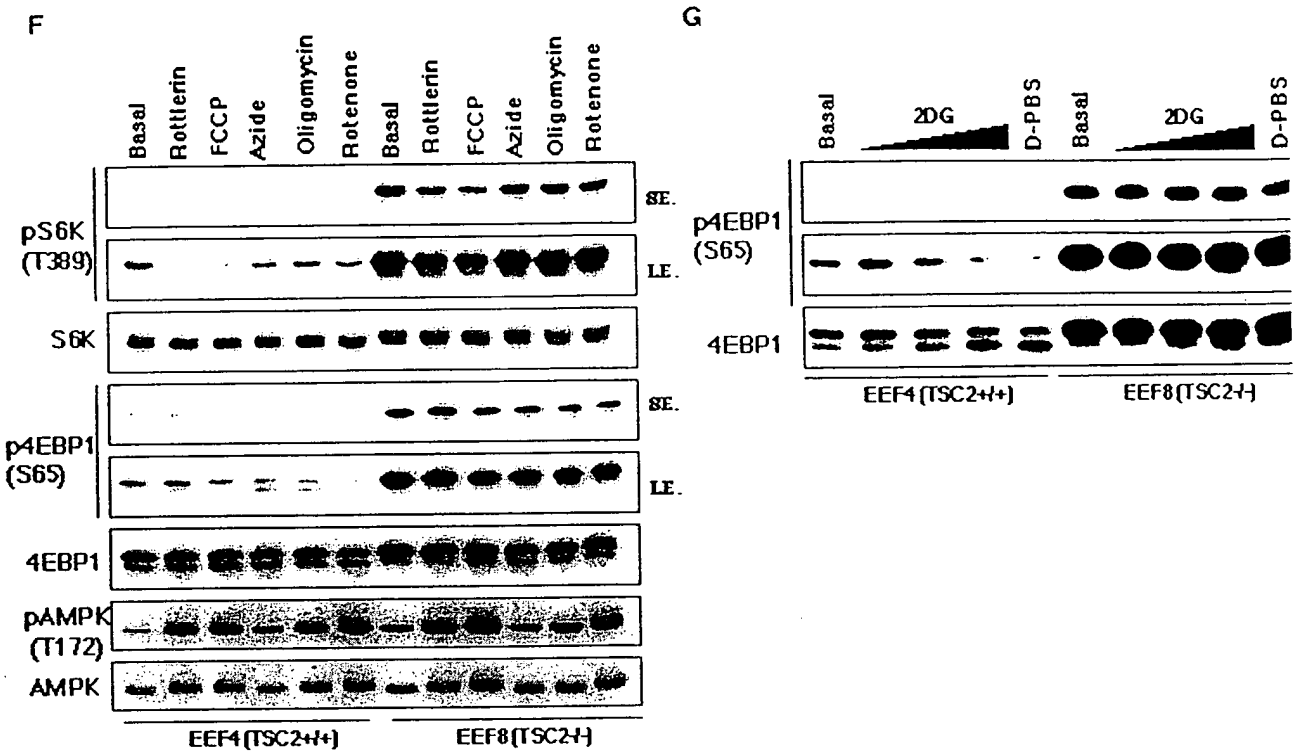


Figure 11

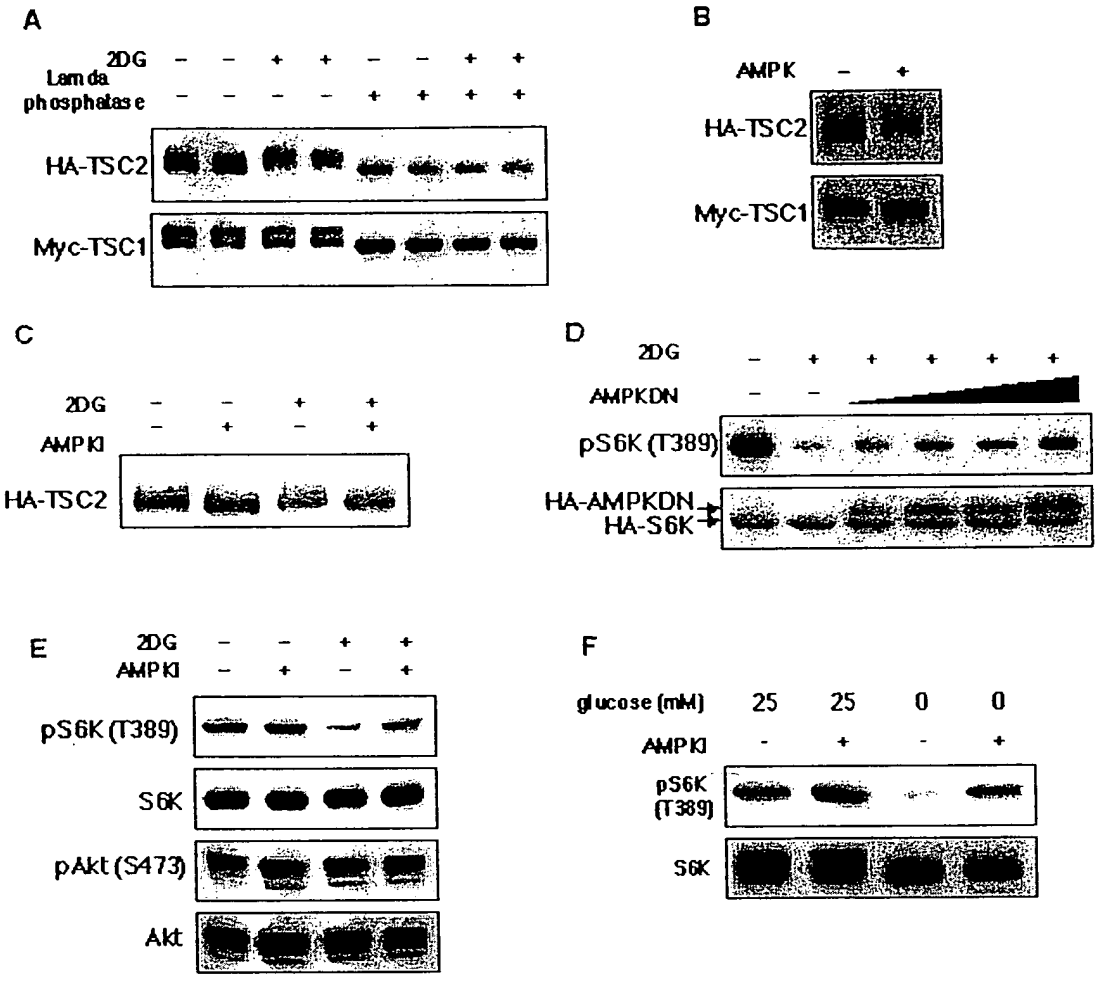

A
2DG
Lamda phosphatase
HA-TSC2
Myc-TSC1
B
AMPK
HA-TSC2
Myc-TSC1
C
2DG
AMPKI
HA-TSC2
D
2DG
AMPKDN
pS6K (T389)
HA-AMPKDN
HA-S6K
E
2DG
AMPKI
pS6K (T389)
S6K
pAkt (S473)
Akt
F
glucose (mM) 25 25 0 0
AMPKI
pS6K (T389)
S6K

Figure 12

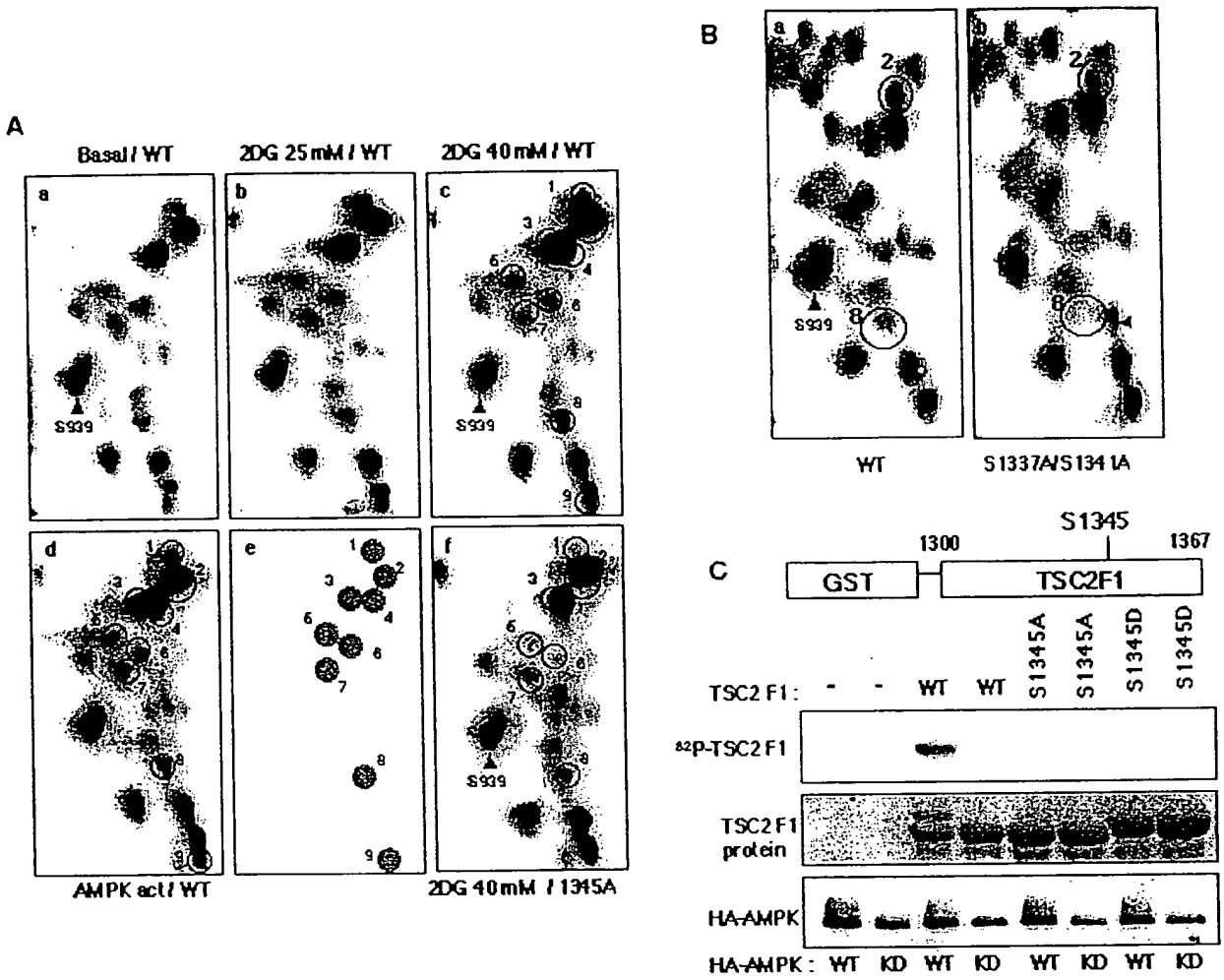
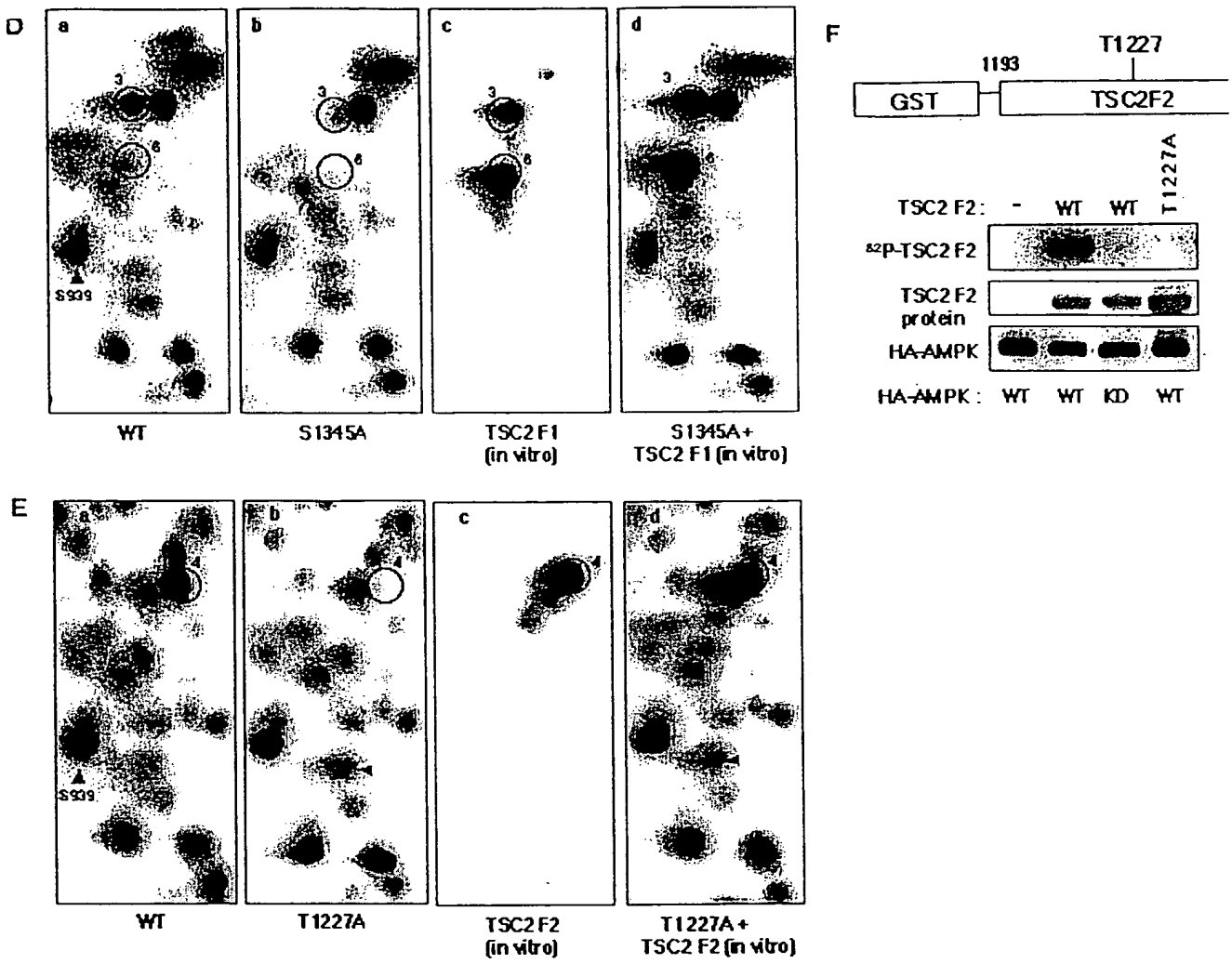

A
Basal / WT
2DG 25mM / WT
2DG 40mM / WT
S939
AMPK act / WT
2DG 40mM / 1345A
B
S939
WT
S1337A/S1341A
C
S1345
1300
1367
GST
TSC2F1
S1345A
S1345A
S1345D
S1345D
TSC2 F1 : - - WT WT
32P-TSC2 F1
TSC2 F1 protein
HA-AMPK
HA-AMPK : WT KD WT KD WT KD WT KD

Figure 12 continued



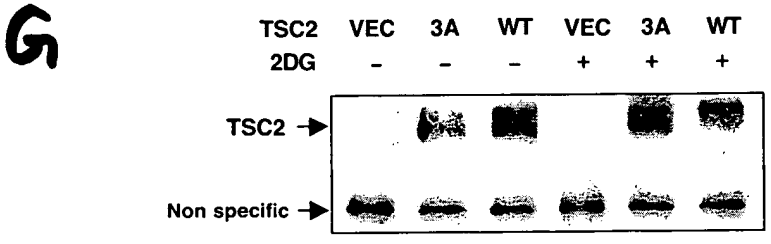


Figure 13

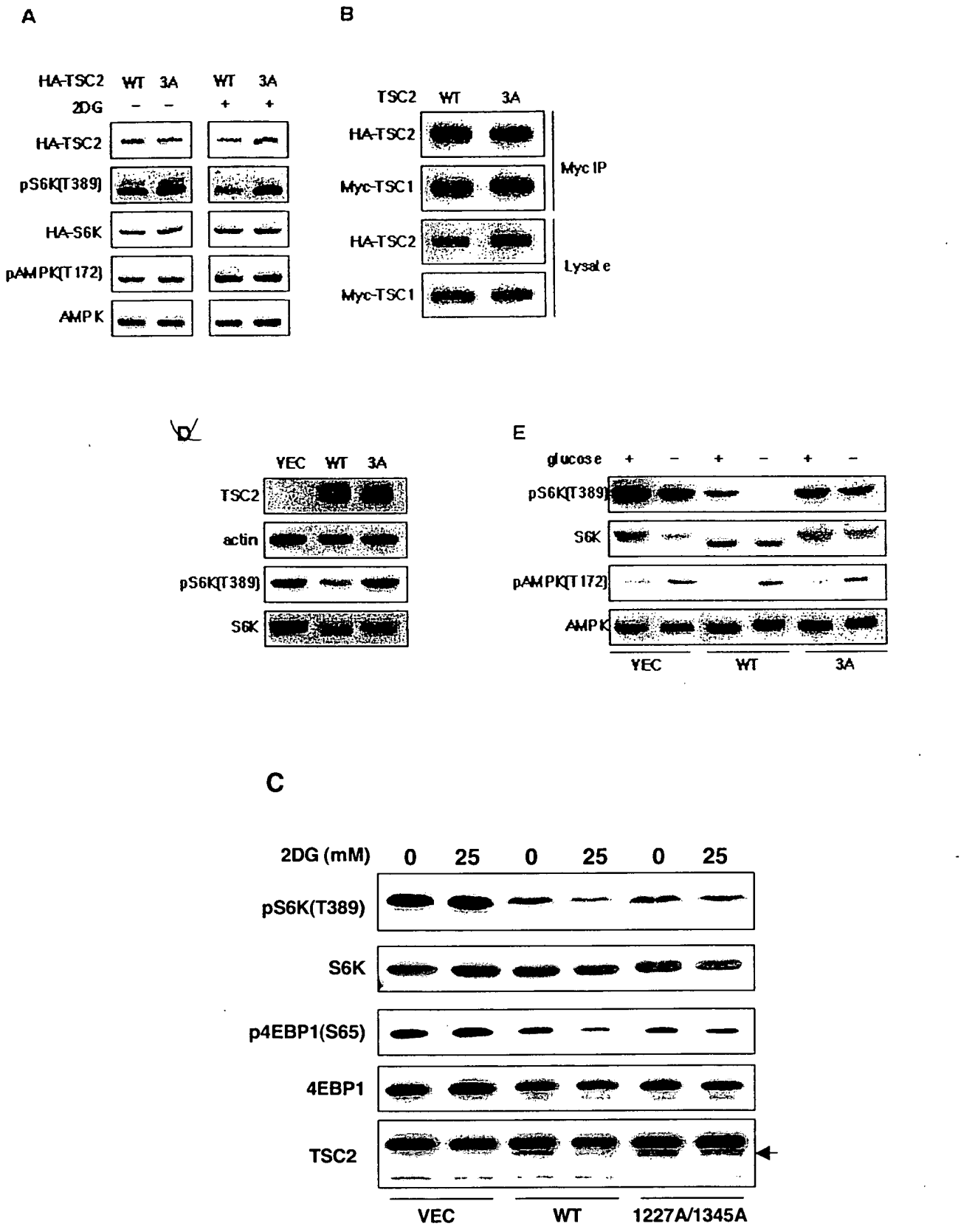

A
HA-TSC2 WT 3A WT 3A
2DG - - + +
HA-TSC2
pS6K(T389)
HA-S6K
pAMPK(T172)
AMPK
B
TSC2 WT 3A
HA-TSC2
Myc-TSC1
Myc IP
HA-TSC2
Myc-TSC1
Lysate
D
VEC WT 3A
TSC2
actin
pS6K(T389)
S6K
E
glucose + - + - + -
pS6K(T389)
S6K
pAMPK(T172)
AMPK
VEC WT 3A
C
2DG (mM) 0 25 0 25 0 25
pS6K(T389)
S6K
p4EBP1(S65)
4EBP1
TSC2
VEC WT 1227A/1345A

Figure 14

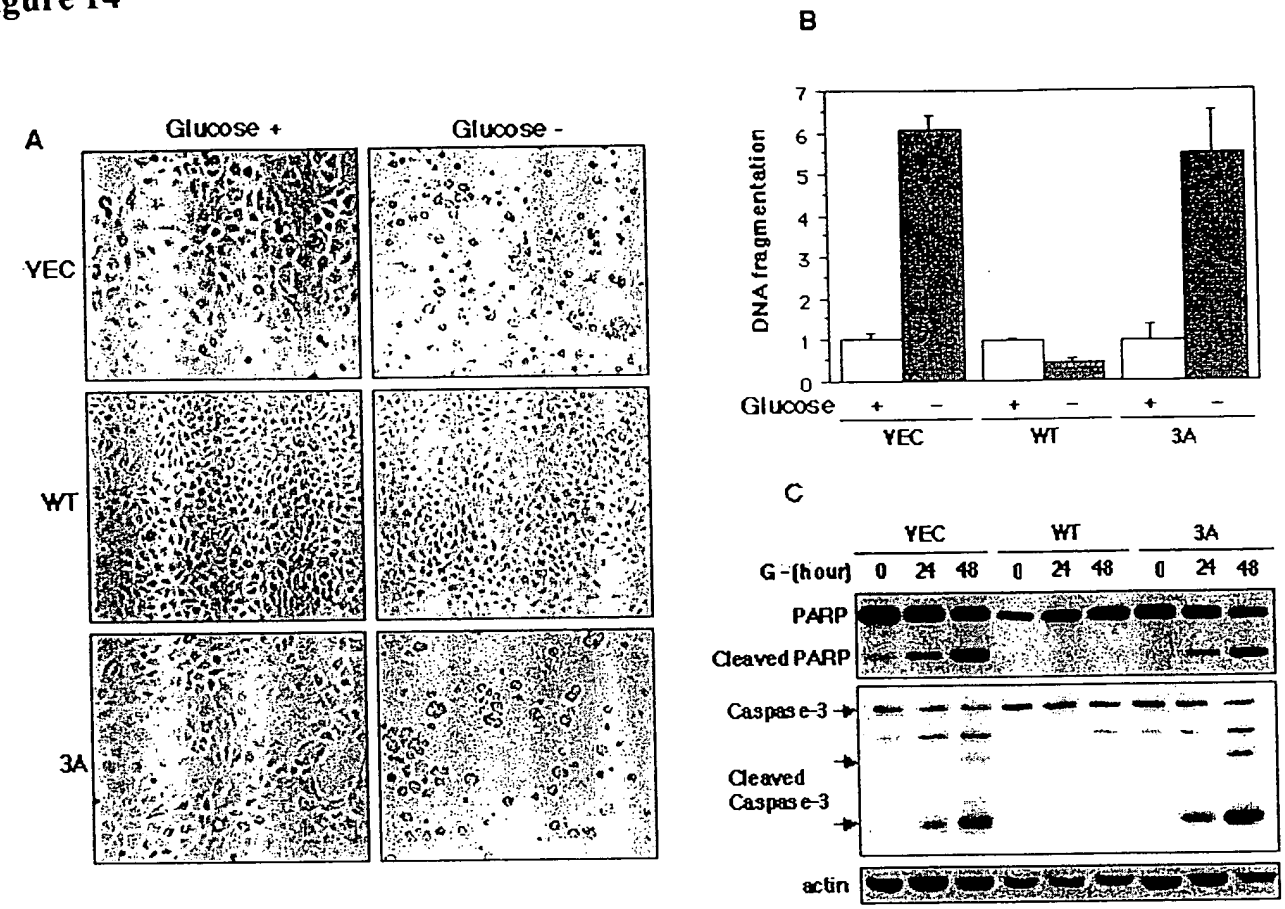

A
Glucose +
Glucose -
YEC
WT
3A
B
DNA fragmentation
7
6
5
4
3
2
1
0
Glucose
+
-
+
-
+
-
YEC
WT
3A
C
YEC
WT
3A
G-(hour)
0
24
48
0
24
48
0
24
48
PARP
Cleaved PARP
Caspase-3
Cleaved Caspase-3
actin

Figure 14 continued

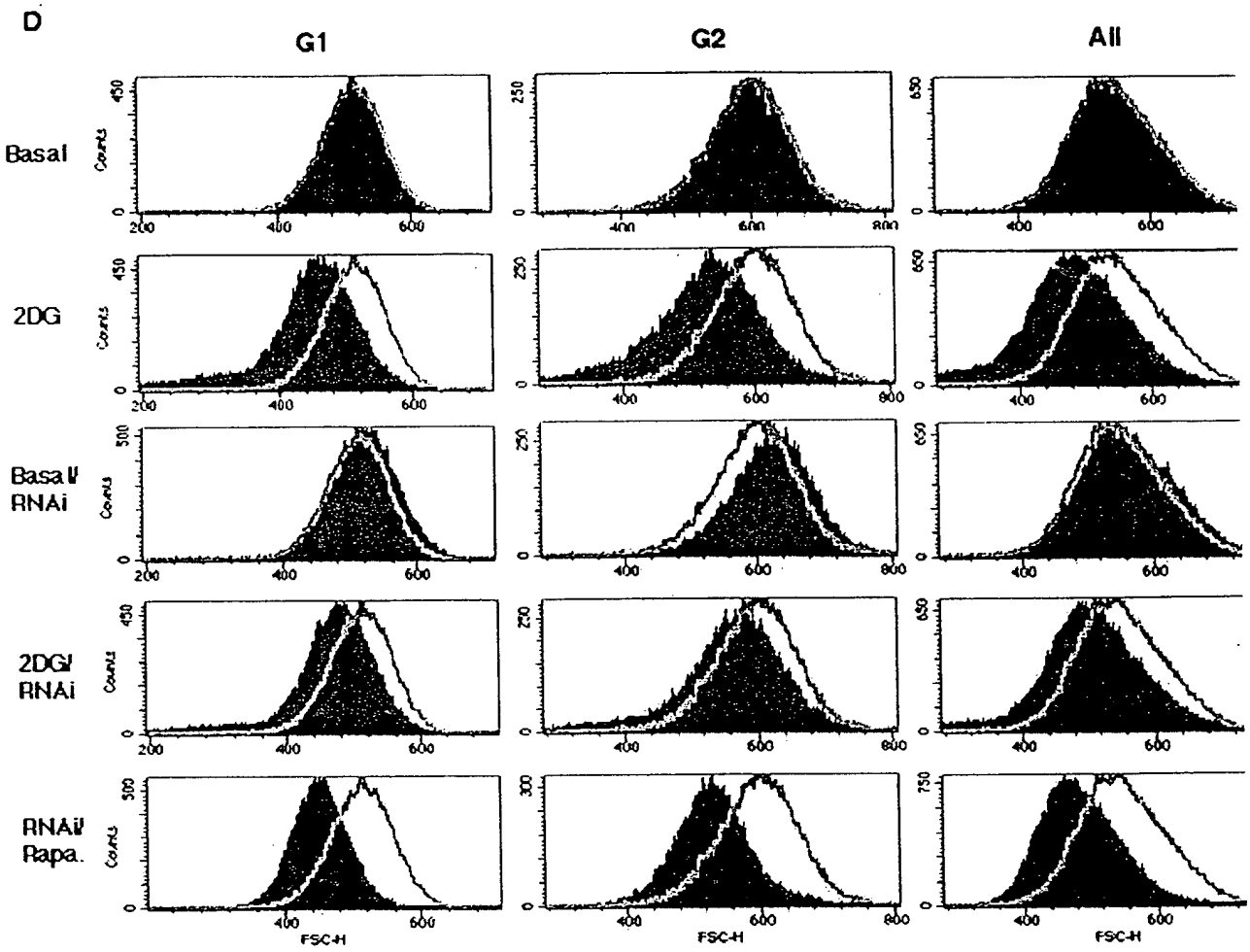


Figure 14 continued

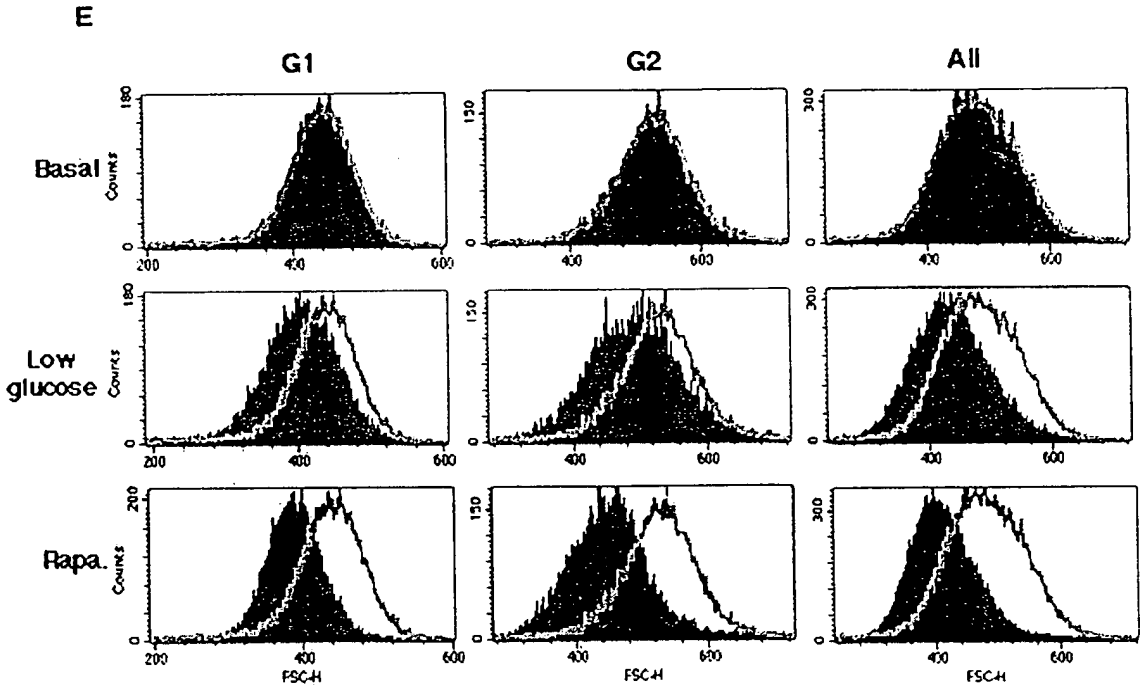
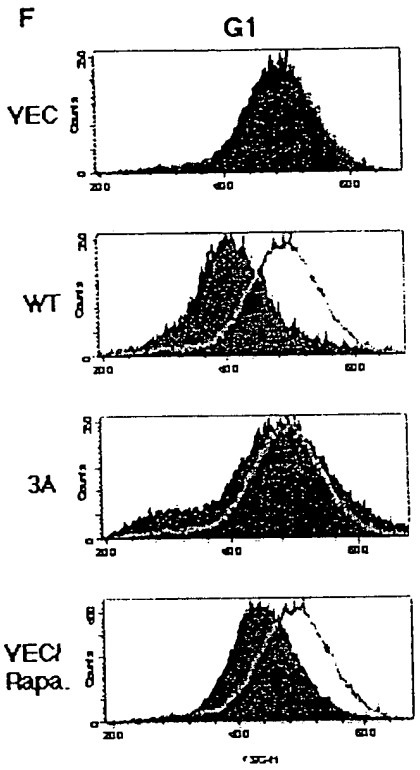
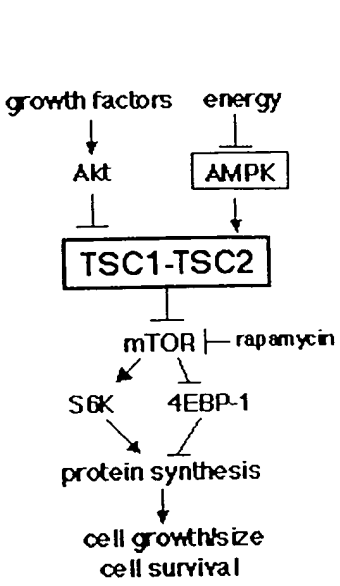


Figure 14 continued





Hyd-(X,Bas)-x-x-**Ser/Thr**-x-x-x-Hyd

Figure 15A

ACC1	ISSLQDGLALHIRSSM**S**GLHL
ACC2	TTGEAETRVPTMRPSM**S**GLHL
HMGR	ALAAGHLVKSHMIHNR**S**KINL
PFK2	TNNFPKNQTPVRMRRN**S**FTPL
TSC2(T1227)	VPAAGTAKPPTLPRSN**T**VASF
TSC2(S1345)	TVDLSFQPSQPLSKSS**S**SPEL

Figure 15B

TSC2(S132)	LGVLRALFFKVIKDYP**S**NEDL
TSC2(T317)	MALWGAHRLYSLKNSP**T**SVLP
TSC2(T509)	LSHIPEDKDHQVRKLA**T**QLLV
TSC2(S625)	SIRLQAFDFLLLLRAD**S**LHRL
TSC2(S802)	REMVYCLEQGLIYRCA**S**QCVV
TSC2(T1227)	VPAAGTAKPPTLPRSN**T**VASF
TSC2(S1345)	TVDLSFQPSQPLSKSS**S**SPEL
TSC2(S1662)	RKDMEGLVDTSVAKIV**S**DRNL

Figure 15C

Figure 16A

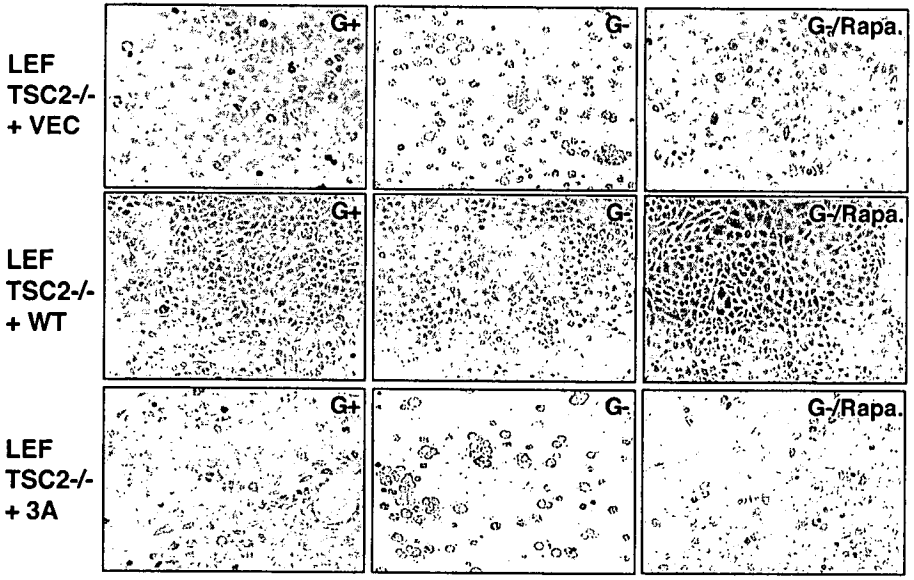


Figure 16B

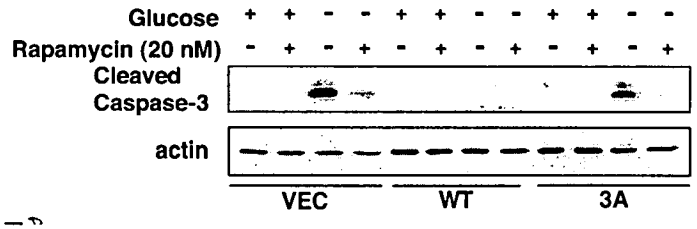


Figure 16C

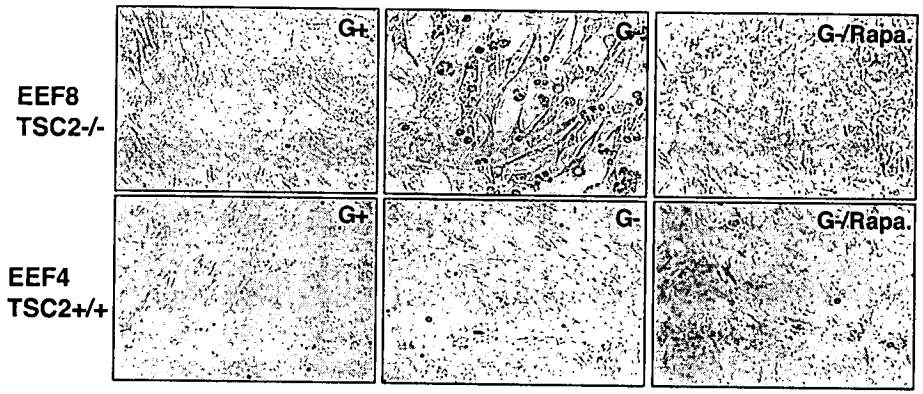


Figure 16D

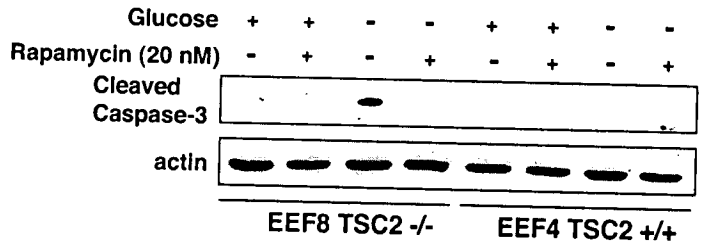


Figure 16E

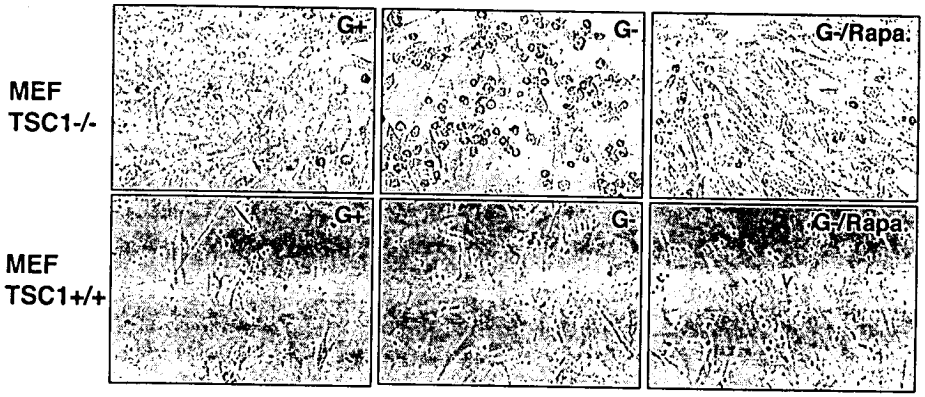


Figure 16F

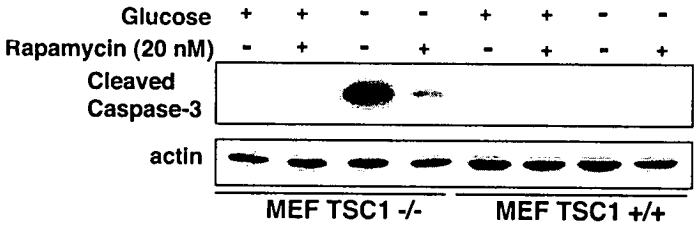


Figure 17A

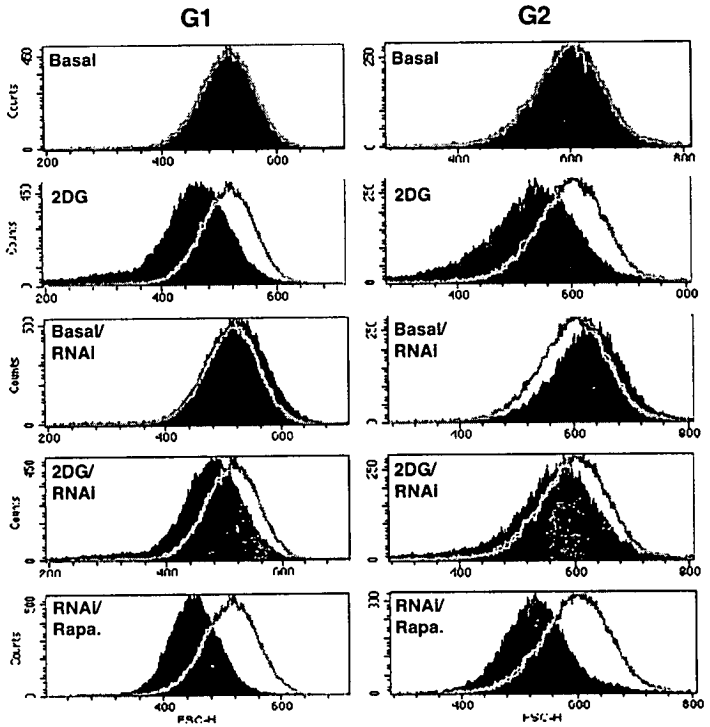


Figure 17B